REVIEW ARTICLE

Laccase Enzyme in Nanotechnology and their Environmental Applications

P. Srinivasan, P. Thiyagarajan, R. Yuvarajan, T. Selvankumar*
PG & Research Department of Biotechnology, Mahendra Arts and Science College (Autonomous), Kalippatti, Namakkal - 637501, Tamil Nadu, India

ABSTRACT: Laccases are ligninolytic enzyme which belongs to the blue multicopper oxidases and participates in the degradation of polymers, and band cleavage of composite aromatic compounds. At present, laccases are commercially produced form bacteria, fungi and plants. These enzymes have wide applications in paper and pulp industries, food industry, textile industry and pharmaceutical industries. Laccases also impart in soil bioremediation, removal of toxic substances like herbicides, pesticides and dye degradation. Due to the wide range purpose, industries have more attention to the isolation and identification of high laccase yielding organisms to fulfil the demand of the enzyme needs. Hence, the present review is to emphasize the properties and applications of laccase in industrial and environmental applications for the removal of pesticides, dye decolorization, phenols and non-phenolic degradation of environmental pollutants.

KEYWORDS: Enzymes, Environment, Immobilization, Industrial applications, Microbes, Laccase.

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1. INTRODUCTION

Enzymes are the biocatalysts playing an important role in accelerating chemical reactions in all stages of metabolism of the living organism. The enzymes particular attention on are called substrates, and the enzyme converts the substrates into diverse molecules known as products. The microbial enzyme research has regularly been updated and increased significantly in the 21st century; studies on their screening, stability, characterization of properties, production on bench-scale to pilot-scale and their application in bio-industry have continuously progressed. Many of the enzymes from microbial sources are already being used in various industrial intermediates. Selected microorganisms including yeast, bacteria and fungi have been globally studied for the synthesis of various enzymes for commercial applications [1]. A large number of industrial need enzymes have been optimized and designed with the input of biochemical engineering, protein engineering, and metagenomics [2].

Laccases (benzenediol: oxygen oxido reductase, EC 1.10.3.2) belong to a large group of enzymes called polyphenol oxidases including multi-copper atoms in the catalytic core and are generally called multicopper oxidases. Three types of copper atoms exist in these enzymes, one of which is accountable for their distinctive blue color. Classically laccase-mediated catalysis occurs with the reduction of oxygen to water adjunct by the oxidation of the substrate. Laccase enzyme productions are broadly disseminated in higher plants and fungi and have also been found in bacteria and insects. In plants Pears, cabbages apples, potatoes and other vegetables is possessed laccase enzyme. They are used for many biocatalytic applications in various biotechnological and industrial domains which have been included biosynthesis of intermediates, improving fibers properties, energy exploitation, bio-detection, environmental protection, degradation and assimilation of synthetic dyes, printing and dyeing industry, bio-pulping in the paper industry, separation of aromatic compounds [3], and degradation of phenols compounds it causes cancer and teratogenicity which was in wastewater. Also, Laccases are used as toothpaste, detergent, soap, mouthwash and diapers in cosmetics such as deodorants for these fast-moving consumer goods, in beverage and food industry for the preparation of wine and juice stabilization characterization [4-6], in dough or baked products to increase the strength of gluten structures; in pharmaceutical industries as anesthetics, anti-inflammatory drugs, antibiotics, and sedatives; and in nanobiotechnology as nanoparticles based biosensors.

Recently, laccases enzymes are not able to exploit full growth efficiency under harsh environmental conditions. Also, wild novel strains which have been tolerated in harsh conditions and produced the highest laccase enzyme with minimum energy consumption in the process are

*Corresponding Author: selvankumar75@gmail.com
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in huge advantages with higher demand. Biotechnological applications of laccase have been extended from laccase-mediator systems, which have provided the ability to oxidize non-phenolic compounds that are otherwise hard or not oxidized o reduced by the enzyme lone.

The flourishing application of laccases in declared areas would necessitate the production of maximum quantity at minimized costs. Abundant production approaches can be adopted as well as media and process optimization to accomplish enhanced process economics. At the same time, over a phrase or disparity expression of laccase in suitable host organisms *Bacillus sp.* would provide means to achieve high titers. Use of inducers such as apple pomace, yeast extract and copper could also enhance production capabilities [7]. The present review is making an effort to provide collective data on various aspects of laccase production and also describes the structure and characterization, catalytic mechanisms and application of laccase industrial and environmental applications

1.1. Sources of laccases

1.1.1. Bacteria

Laccase producing bacterial species was first discovered in rice rhizosphere and identified as *Azospirillumlipoferum*[8].Later, laccase has been identified from a broad range of bacterial species in various genera [9-25]. Bacterial laccases are more prevalent in industrial and environmental applications due to their availability, flexibility, thermostability, alkalineoptimum and radiance tolerance. Moreover, it has low redox potentials and also widely used in decolorization of dyes, bioleaching of paper and pulp industries [26, 27].

1.1.2. Fungi

Laccase production has been reported in many fungal species. The premier laccase producing fungi are *Ascomycetes* and followed by *Zygomycetes* and *Chytridiomycetes*. The genus *Ascomycetes* are phytopathogens and isolated from soil, freshwater, wood logs on degradation. The laccase enzyme producers are more prevalent in fungi than plants. Fungal laccases are noticed in together intra and extra-cellular physiological processes of pathogenesis, delignification, pigmentation, and morphogenesis [28]. Recently, researchers are focus on screening of laccase producing novel fungal wild species and researched developing higher yield stains to meet out the demands of industries. The higher yield wild fungal laccase producers are *Basidiomycete* families. The researchers are carried out on the wild strains critically and carried out the experiments on genetic material sequences; phylogenetic relationship, catalytic property, and expression regulation of laccase isozymes systems [29,30].

1.1.3. Plants

The plant laccase was first isolated from the Japanese lacquer tree *Rhusvernicifera*. They are generally spread in a wide range of upper plants. The plant laccase enzyme activities have been pragmatic in trees, apples, turnips, cabbages, potatoes and asparagus [31]. These enzyme gene families in plants are still better than fungal laccase families. For case, rice (*Oryza sativa*) found to have at slightest 22 laccase genes [32]. *Arabidopsis thaliana,* the model plant has 17 gene members in its laccase family, which play many roles in plant enlargement and progress, depend upon the mutant characterization and expression profiling [33].

1.1.4. Insects

Insect laccases are the least characteristic of all identified laccases. Insect laccases have an important role in cuticle sclerotization and melanization of the physiological functions of the insect life cycle [34-37].

1.2. Enzyme production by fermentation

The large-scale production of laccase enzyme could be achieved by fermentation processes. They are i) Submerged fermentation and ii) Solid-state fermentation.

1.2.1. Submerged fermentation

Submerged fermentation process contains the microbial growth in a liquid medium supplemented with nutrients in aerobic conditions. The manufacturing production of enzymes is mainly achieved by submerged cultivation. The perfect of nutritional and ready conditions is the first step to be intense to achieve high production. Waste and cheap raw materials from many resources are used in huge amounts can be employed in submerged cultivation. These resources have important concentrations of all the soluble elements and even inducers for laccase production [28]. The natural raw materials are the nutrient sources in submerged fermentation, which cause glutinousness nature of production medium and subsidize the production and downstream process by hampering impeller action causing obstructions consequential in restrictions of oxygen and mass transfer [33].

1.2.2. Solid-State fermentation

SSF is a suitable method for the industrial enzyme production using natural substrates such as agricultural residues because they impersonate the environmental surroundings under which the fungi grow in nature. Laccase enzyme production

makes the most of many agricultural wastes as substrates such as pearl millet, finger millet, grape seeds, grape stalks, cone, cotton stalk, barley bran, molasses and wheat bran [37]. As compared to the additional substrates, rice bran found to produce maximum laccase enzyme by SSF [18]. Solid substrate cultivation is cost-effective as well as low-cost substrates are being utilized for enzyme production. However, there are few disadvantages or limits for growing microorganisms in SSF that includes the minimal transfer of oxygen, nutrients, moisture, and temperature and pH regulation due to the absence of any established fermentor designs [26].

1.3. Optimization of enzymes production

Optimization is one of the major strategies for obtaining a maximum yield of industrial enzymes within the limiting factors. The high yield strains are subjected to optimize with various parameters for an optimal yield of products. In laccase enzyme, the high yield stains are designed with molecular cloning techniques and the improved strains are subjected to optimization of media, environmental, physical conditions and stress conditions. The industrial production of laccase enzymes is highly influenced by media components and other conditions such as temperature, pH and agitation [38]. The optimization procedure gives enough information to scale-up the fermentation process in design and optimizing of the suitable fermentation medium with medium nutrient composition, product concentration, yield and volumetric productivity for industrial applications. It has been well documented that media components and physical factors greatly influence the production of enzymes in microorganisms and their interaction plays a significant part in the biogenesis of these enzymes.

Media optimization is frequently done to retain an explicit ratio among various medium components, however, it was completely utilized by fungi or bacteria on other hand obtain a cost-effective and prevent the wastage of medium components, while metabolite yield at the closing stages of fermentation. There are many experimental strategies available for optimization of enzymes and metabolites production from microbial sources. Optimization of media compounds by fixed "one-variable-at a-time" strategy by optimization of fermentation media compounds by traditional involving changing one independent uneven on a point in time, most commonly used act in fermentation technology. This plan varies in time and costly as soon as a massive number of factors variables are measured and is unable of detecting the true most favorable, due mainly to the connections amongst the factor [39].

1.3.1.Response Surface Methodology (RSM)

RSM is a group of statistical and numerical techniques helpful for increasing, humanizing and optimizing process in which a reaction of attention is partial by some variables and the purpose is to optimize this response. RSM has important appliance in the design, advance and preparation of novel industrial enzyme yield, as well as in the development of open product design process. Processes define the most efficacies of the free variables, only or here mixture. In accumulation to analyze the special effects of the free variables, this experimental method generates a numerical model which describes the chemical compound or biochemical processes [40-43].

RSM is usually achieved by real-time testing of many factors in the limited numeral of experiments. In data, the purposeful report among the needy and the free variables can be firm by using regression investigation which also explains the significance of the variables [39].

1.3.2. Purification of laccase

Generally, plant laccases are extracted and purified from tissues. Whenever the bacteria are utilized for the laccase production, the extracellular enzyme extracted from the medium followed by the purification process. Two operations were followed for the complete down-streaming process, in which primary operation is solid/liquid medium segregation by filtration and centrifugation. This step will be followed by dewatering, which can be carried using the salting-out precipitation method with ammonium sulphate salt [44]. The second stage of enzyme purification is achieved using ion exchange and gel filtration chromatographic techniques. Purification may be a single or multi-step process. Other works include purification of laccase from *Bacillus* sp. with ethanol precipitation, Phenyl Sepharose, DEAE-Sepharose. After separation, enzyme processing is carried out through immobilization techniques.

1.3.3. Immobilization

The term 'immobilized enzymes' refers to 'enzymes physically attached to an inert assured definite area of space with preservation of their catalytic activities, and which can provide increased conflict to change in surroundings much as pH or temperature. Besides a more easily convenient handle care of the enzyme, it also substantially simplifies the manipulation with the biocatalytic reaction and the control of the preservation process [45] while enhancing the enzyme stability under both storage and

operational conditions. Immobilization provides a facile separation of the enzyme from the product [46-48], hence protein contamination of the product is minimized or avoided altogether. Apart from the easy separation of the enzyme from a mixture of sodium alginate solution with calcium chloride, enzyme immobilization (entrapment) also remarkably reduces the cost of enzyme and the enzymatic immobilized products. Insolubilization of the enzyme by attachment to a matrix also imparts several added benefits such as; restrict the freedom of movement of an enzyme from the reaction solution and value of enzyme stability against solvents, pH, temperature, contaminants and impurities. Which is also helps for the highest recovery and reuse of more active enzymes and enable their application in continuous fixed-bed operation in fermentation. It is possible to conclude that enzyme immobilization increases the productivity of the biocatalysts and enhances their features, making them more attractive for diverse applications.

1.4. Laccase Immobilization

Laccases are immobilized for various reasons including recycling, stability during the operation process, and resistance to application conditions. The factors responsible for activity improvement depend upon different factors nature of the laccase enzyme, desired method of immobilization and preparation parameters. The immobilized microbial laccases are more broadminded to high temperatures, storage and can be reused multiple times when compared with their free enzyme counterparts [49, 50]. They are also found to be more resistant to inhibitors such as NaCl. Even though there are some limitations such as reduced enzyme flexibility, and diffusion limitations, immobilization method can improve the catalytic activity of microbial laccases occasionally.

1.4.1. Magnetic metal nanoparticles

Magnetic metal nanoparticles have been utilized in enzyme immobilization because of their precise properties together with superparamagnetic, excessive surface area, huge surface-to-quantity ratio, and easy separation under outside magnetic fields [51]. Another key issue to take complete advantages of nanoparticles such as MNPs is the way to ideally modify the orientation of the enzymes on the supports. Compared to porous supports, non-porous nanoparticles have no external diffusion problems, making them more competitive especially for large scale industrial usage in solid-liquid systems (e.g., precipitated protein). The frequently utilized MNPs are iron oxides, among which superparamagnetic Fe_3O_4 nanoparticles are the most prevalent materials because they have low toxicity, good biocompatibility [32,33].

1.4.2. Preparation of magnetic nanoparticles

MNPs can be produced by physical, chemical and biological methods: (i) physical methods [45,46], such as gas-phase evidence and electron beam lithography; (ii) dripping chemical preparation methods, such as sol-gel synthesis [52], oxidation method [53,54], chemical co-precipitation, hydrothermal reactions [55], flow injection synthesis [56], electrochemical method, aerosol/vapor phase method sonochemical breakdown reactions [57], the supercritical fluid method [58], synthesis using nanoreactors [59] and (iii) microbic methods [60,61]. Since the physical and chemical nature has decisive results at the applicability of magnetic nanoparticles, comprehensive surface characterization strategies are utilized for higher expertise of the surface properties together with chemical composition, surface morphology, and spatial distribution of the functional groups.

1.4.3. Enzyme immobilization methods

Traditionally, the selection of an appropriate immobilization approach is a very crucial part of the immobilization process as it performs the largest role in determining the enzyme activity and characteristics in a selected response. Immobilization techniques can be divided into two major characterizations namely, the chemical and physical methods. Although, there are four main principal techniques used for the immobilization of enzymes specifically, Adsorption, Entrapment, Covalent and Cross-linking [53].

1.5. Applications of Laccase

In the last few decades, enzyme-like laccases have much important attention from many researchers due to their ability to highly recalcitrant environmental pollutants and oxidize both phenol, non-phenol lignin related compounds. One of the main applications of detoxification of dye-containing industrial effluents from textile and leather industries by laccases, on the other hand over pulp bioleaching, and use as a tool in the pharmacy industry and as a bioremediation agent to dirt-free up herbicides, pesticides and certain explosive in soil [58] [Fig.1]. Water purification systems laccases are also used as clean-up agents, as catalysts for the manufacture of anti-cancer drugs and even as ingredients in cosmetics. Laccases also find application in the food industry that includes beverage (fruit juice, wine and beer) processing, sugar beet pectin gelation, baking etc.

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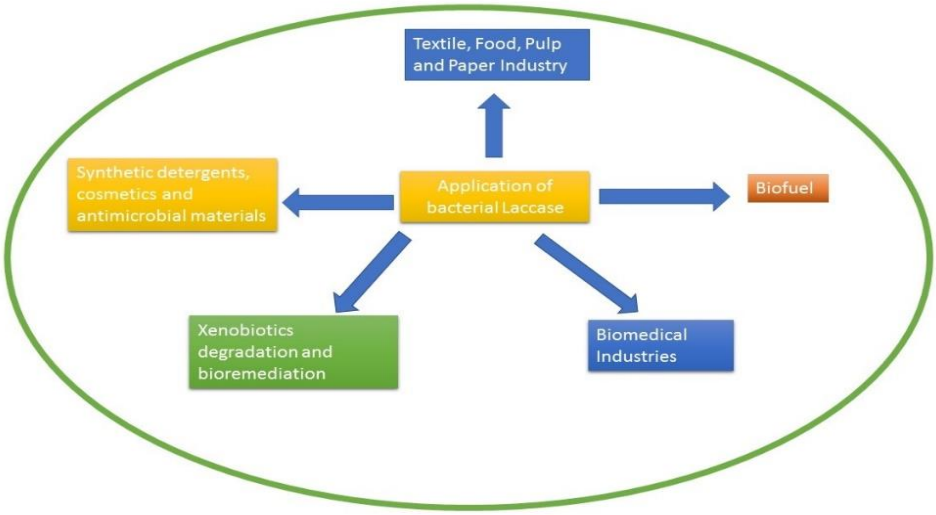

Fig.1. Applications of bacterial laccase

1.5.1. Bioremediation

The association of laccase in biodegradation was specifically because of its catalytic properties. Laccase is used for decolorizing dye residence effluents which capacity is rarely decolorized from side-to-side conventional dirt behavior foliage. The xenobiotic compound present in contaminated soil, polycyclic aromatic hydrocarbons (PAHs) in natural oil deposits and fossil fuels have been easily degraded utilizing laccase. Laccase becomes determined to be dependable for the change of 2, 4, 6-trichlorophenol to 2, 6-dichloro-1, 4-hydroquinone and 2, 6-dichloro-1, 4-benzoquinone. Laccase arbitrate system has been also used to oxidize dibenzothiophene ethyl-carbazole, carbazole, fluorine and alkenes [57, 58]. LMS has been considerably used for the oxidation of recalcitrant polycyclic aromatic hydrocarbons such as naphthalene, having two aromatic rings, and the three-ring compounds anthracene, phenanthrene and several other contaminants [54].

1.5.2. Dyedecolorization

The application of laccases in dye decolourization has enlarged in modern years and many studies have been confirmed for dye decolourization using both crude and purified forms of laccase. Some of the portion of the organic compound as redox mediators facilitate the laccase dye degrading activity and enhance its specificity to a wide range of synthetic dyes [38]. Laccases have been used for the decolorization of dyes belonging to different categories such as azo, anthraquinone, heterocyclic, triphenylmethane dyes etc. Many studies were carried for the degradation of azo dyes by microbial laccases [58].

1.5.3. Pharmaceutical Applications

The application and stability of laccases in the pharmaceutical industry have been exploited due to their specificity and bio-based nature suggested that microbial laccases may be utilized for the synthesis of the drug of anti-inflammatory, anesthetics and sedatives [45]. Pharmaceuticals and Personal Care Products (PPCPs) are detected in municipal wastewater. Laccase-catalyzed reactions are employed for the removal of PPCPs [62]. Laccase from *Myceliophthora thermophila* is used in the degradation of several anti-inflammatory drugs (diclofenac and naproxen) and estrogen hormones. Laccase from *Clitocybe maxima* exhibited anti-proliferative activity against Hep G2 and MCF-7 tumour cells [65]. Laccases are used as markers in immunological, histochemical and cytochemical assays by covalent conjugation with binding molecules.

1.5.4. Phenol removal

Removal of phenols and phenolic compounds in aqueous effluents release from industry is an important practical problem for the environment, therefore virtually all phenols are toxic and their presence in several industrial wastewaters is a health hazard [45]. The use of free laccase, tyrosinase and peroxidase, which catalyse the oxidative coupling of phenol compounds resulting in the formation of water-insoluble oligomeric and polymeric products which are then removed by sedimentation or filtration, has been proposed [48].

1.5.5. Pulp bioleaching

The major problem encountered in the pulping process is the characteristic brown color of the pulp due to the presence of residual lignin [54]. The chemical processes are usually employed in the industries for the removal of lignin and hemicelluloses from the pulp thereby the brightness of the pulp is achieved. Although the chemical treatments are an effective method to achieve this task, the high cost and related pollution harms make them unattractive [56]. In this method is the eco-friendly treatment of pulp that involves either microorganisms or their enzymes called Bioleaching. This process helps in the selective removal of lignin and hemicelluloses components without degrading cellulose. Cellulases and xylanases are the most widely used microbial enzyme for bioleaching however, the role of laccases in the process has also been elucidated in the recent year's application [57].

1.5.6. Paper industry

In the industrial preparation of the paper, the degradation and separation of lignin in wood pulp are conventionally obtained using oxygen or chlorine-based chemical oxidants. Enzymatic treatment of method like non-chlorine bleaching of pulp obtained brighter pulp with low lignin content [60]. Since wood and other soil materials are naturally degraded by biological origin, the use of lignin-degrading enzymes of laccases would provide a new alternatives approach in pulp and paper industries. Employing microbial laccase in lingo cellulosic fibers will improve the physical properties and chemical of Kraft pulp fibre products [61]. Bacterial laccases from *Streptomyces cyaneus* CECT 3335 and *Pseudomonas stutzeri*have been examined for bio-bleaching of eucalyptus kraft pulps using HOBT (Hydroxybenzotriazole) and ABTS as redox mediators.

1.5.7. Nanotechnology

The ability of laccases to catalyze electron transfer reactions without additional cofactors is being employed in biosensors. Biosensors also containing laccase have been developed for immune, histochemical and cytochemical assays used for the determination of glucose, aromatic amines and phenolic compounds. Laccases are immobilized on various substrates composed of pyrolytic graphite, ceramics supports and carbon fibres which can be used as electrodes [62]. The redox potential of laccases is also utilized in the biofuel cell to provide power for small transmitter systems. Biofuel cells do not require proton exchange membrane like in a traditional fuel cell due to the specificity of the laccase enzyme. Coupling the laccases with nanoparticles can further increase the laccase activity in the biofuel cells [63]. Laccase-based biofuel cell is also used in therapeutic applications for providing power to cells source implanted in a human body.

CONCLUSION

Laccases are highly versatile enzymes and are involved in the degradation of a range of complex and recalcitrant compounds and thus find application in an extensive range of industries. The biotechnological importance of laccases has led to a drastic boost in the demand for these enzymes and oriented the interest of scientists towards the same in recent years. The present study thus focuses on the isolation of laccase producing organisms and large-scale productions of laccases using inexpensive sources like agro-wastes. In this regard, inexpensive raw wastes will be explored for maximizing laccase production under submerged and solid-state fermentation. It is also described the optimization procedures and catalytic properties of laccase enzyme with immobilization with magnetic nanoparticles. This review also highlights the potential use of pharmaceutical applications and cleanup of environment for the removal of pesticides, dye decolourization, removal of phenolic compounds, paper and pulp industries. This review helps to understand the properties of laccase enzymes in nanotechnology for biosensor and biofuel cell development.

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